Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-~~Report~~ of the in-house investigations of **Diacetyl Tartaric Acid** in the developing chicken embryo 12/29/77

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335

DATE: December 29, 1977

Through: HFF-150 _____

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FROM : Supervisory Chemist
Whole Animal Toxicology Branch (HFF-155)

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the inhouse investigations of Diacetyl

Tartaric Acid in the developing chicken embryo.

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Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Diacetyl Tartaric Acid

Protocol:

Diacetyl Tartaric Acid (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in chloroform as the solvent by two routes and two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 gave the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Columns 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square Test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Diacetyl tartaric acid showed little toxicity when administered up to 500 mg/kg. Yolk treatment at 0 hours resulted in a calculated LD_{50} of 171.86 mg/kg (8.59 mg/egg). No LD_{50}'s could be calculated for the other three test conditions.

There were a few scattered abnormalities observed throughout the four test conditions, but the anomalies were not different from nor significantly higher in incidence than those observed in the background. Diacetyl tartaric acid displayed no teratogenicity under the test conditions employed.

1. Diacetyl Tartaric Acid, Witco Chemical Co. Lot # HP-308

2. McLaughlin, J., Marliac, J. P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol.

3. Verrett, M. J., Marliac, J. P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002-1006

4. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I

Diacetyl Tartaric Acid Air Cell At 0 Hours Table 1

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
25.00	500.00	104	52.88	3.84	0.00
12.50	250.00	105	60.95	3.80	1.90
5.00	100.00	105	56.19	5.71	2.85
2.50	50.00	105	46.66	4.76	5.71
1.250	25.00	105	47.61	3.80	0.95
Chloroform		95	47.36	3.15	1.05
Controls		319	14.73	0.00	0.00

**Slope not significantly different from zero p 0.05

Diacetyl Tartaric Acid
Air Cell at 96 Hours

Table 2

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
1.00	20.00	110	65.45	5.45	3.63
0.50	10.00	110	63.63	5.45	6.36
0.20	4.00	110	73.63	4.54	4.54
0.10	2.00	110	80.00	2.72	2.72
0.050	1.00	109	76.14	5.50	2.75
Chloroform		115	70.43	2.60	1.73
Controls		319	14.73	0.00	0.00

**Slope is negative

Diacetyl Tartaric Acid Yolk at 0 Hours

Table 3

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
25.00	500.00	105	75.23*	1.90	0.95
12.50	250.00	105	72.38*	0.00	0.00
5.00	100.00	105	65.71*	0.95	0.00
2.50	50.00	105	63.80*	1.90	0.95
1.250	25.00	105	55.23*	0.00	0.00
Chloroform		95	37.89	2.10	2.10
Controls		319	14.73	0.00	0.00

*Significantly different from solvent $p \leq 0.05$

**LD_{50} 171.861 mg/kg (8.593 mg/egg)

Diacetyl Tartaric Acid Yolk at 96 Hours Table 4

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
1.00	20.00	105	44.76	0.95	0.95
0.50	10.00	105	46.66	2.85	2.85
0.20	4.00	104	40.38	2.88	0.00
0.10	2.00	105	48.57	2.85	0.95
0.050	1.00	105	32.38	0.95	0.95
Chloroform		110	35.45	0.90	0.00
Controls		319	14.73	0.00	0.00

**Slope not significantly different from zero p = 0.05